Asian Journal of Chemistry; Vol. 26, No. 17 (2014), 5816-5820

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.18212

Comparison of Chemical Composition in Decoctions of *Aconitum kusnezoffii* Reichb and *Pinelliae rhizoma* Praeparatum Extracted by Different Methods†

X.Y. ZHAI[1,2], G.L. XU[2], L. ZHANG[2], C. JIN[2], Y.L. FENG[2], B.T. LI[2] and S.L. YANG[1,2,*]

[1]The College of Traditional Chinese Medicine,Beijing University of Chinese Medicine, Beijing 100102, P.R. China
[2]Research Center for Differention and Development of TCM Basic Theory, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, P.R. China

*Corresponding author: Tel: +86 791 87119632; E-mail: slyang3636@126.com

AJC-15800

To find the discrimination of the combination of *Aconitum kusnezoffii* reichb and *Pinelliae rhizoma* praeparatum from the chemical composition, two co-decoctions and two mixed decoctions were extracted by alcohol and water, respectively. Batches of these decoctions were analyzed by UHPLC/Q-TOF-MS. The datasets were analyzed by OSC-PLS-DA. The content of monoester-diterpenoid alkaloids (MDAs) including benzoylaconine (BAC), benzoylmesconitine (BMA), benzoylhypaconine (BHA) in MDW was lower than that in CDW, but the content of amine alkaloids (AAs) that including aconine, mesaconine, hypaconine in MDW was higher than that in CDW. The content of diester-diterpenoid alkaloids (DDAs) including aconitine (AC), mesaconitine (MA) and hypaconitine (HA) in MDA was lower than that in CDA, but 14-benzoyl-8-O-methyl-aconine, mesaconine, BMA, BHA, neojiangyouaconitine in MDA was lower than that in CDA. In both alcohol extraction and water extraction, the chemical composition of toxicity in mixing decoction was lower than that in co-decoction.

Keywords: *Aconitum kusnezoffii* reichb, *Pinelliae rhizoma* praeparatum, Combination, UHPLC/Q-TOF-MS, Chemical composition.

INTRODUCTION

A. kusnezoffii reichb and *P. rhizobium* praeparatum are warm-hot in herbal natural theory. The combination *of A. kusnezoffii* reichb and *P. rhizoma* praeparatum, one of the eighteen incompatible medications (Shi Ba Fan), are regarded as incombination in traditional Chinese medicine theory. The combination of *A. kusnezoffii* reichb and *P. rhizoma* praeparatum is also incompatible in Pharmacopoeia of the People's Republic of China[1]. However, they are used together as an important medicine to treat arrhythmia, headache and pain of shoulder [2]. Moreover, the modern pharmacology researches show that the maximum tolerated dose of the combination of the *Aconitum* and *P. rhizobium* praeparatum is 67.5 g/kg on mice by intragastric administration, equivalent to 150 times of adult dosage[3]. Also the long-term toxicology study on rats indicates the toxicity of compatibility of the *Aconitum* and *P. rhizobium* praeparatum is not obvious[4]. While the underlying mechanisms of the combination were not found.

The toxicity of *A. kusnezoffii reichb* mainly derives from diester-diterpenoid alkaloids (DDAs) that mainly including aconitine (AC), mesaconitine (MA) and hypaconitine (HA). They can be changed into less-toxic monoester-diterpenoid alkaloids (MDAs) including benzoylaconine (BAC), benzoylmesconitine (BMA), benzoylhypaconine (BHA), which play an essential role in reducing the toxicity of *A. kusnezoffii* reichb[5]. AC, MA, HA are the effective ingredients and toxic components of *A. kusnezoffii* reichb[6,7].

UHPLC, with the features of high pressure, high sensitivity, high isolation, has obvious advantages in the separation and analysis of traditional Chinese medicine and other complex systems. Q-TOF-MS is a high resolution tandem mass spectrometry. For the remarkable features of high sensitivity and high selectivity, high quality mass spectra and accurate molecular weight compounds can be acquired. In order to get a better selection and more information, as a good qualitative determination tool, UHPLC/Q-TOF-MS gets its wild application in scientific research field, especially in the traditional Chinese medicine complex system. It is becoming one of the most powerful tools[8]. Meanwhile, UHPLC/Q-TOF-MS combined with multivariate statistical techniques, such as principal component analysis (PCA) and partial least squares discriminate analysis (PLS-DA), is an increasingly popular technique in the field of herbal drug analysis[9].

†Presented at 2014 Global Conference on Polymer and Composite Materials (PCM2014) held on 27-29 May 2014, Ningbo, P.R. China

In this study, UPLC/Q-TOF-MS technology combined with orthogonal signal correction-partial least square discriminate analysis (OSC-PLS-DA) was used to explore the chemical composition of co-decoctions and mixed decoctions of *A. kusnezoffii* reichb and *P. rhizobium* praeparatum by different methods.

EXPERIMENTAL

A. kusnezoffii reichb and *P. rhizobium* praeparatum were purchased from the Chinese medicinal materials Company (Beijing, China) and authenticated by Associate Professor KeZhong Den of the Jiangxi University of Traditional Chinese Medicine. Aconitine (X-015-110425), mesaconitine (X-011-110425), hypacontine (MUST-13011706), benzoylaconine (B-010-110316), benzoylmeaconine (B-009-110316), benzoylhypaconine (B-016-110316) bought from the Chengdu Ruifensi Biological Technology Co., Ltd. (Chengdu, China).

Acetonitrile (HPLC grade) was purchased from the Tedia Company (USA). Deionized water was purified by Milli-Q system (Millipore, Bedford, MA, USA). Formic acid (HPLC grade) was purchased from Dikma technology Inc. (Lake Forest, CA, USA). Leucine-enkephalin was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of the samples

Mixed decoction of water (MDW): 5 g of *A. kusnezoffii* reichb and 5 g of *P. rhizobium* praeparatum was weighted individually and mixed together (1:1), soaked into 100 mL water for 0.5 h and boiled for 1 h. The decoction was filtered through three layers of gauze then 50 mL water was added for the second decoction with a duration of 0.5 h. The filtered and mixed decoction was adjusted to 150 mL.

Co-decoction of water (CDW): 5 g of *A. kusnezoffii* reichb was weighted, soaked into 50 mL water for 0.5 h and boiled for 1 h. The decoction was filtered through three layers of gauze and 25 mL water was added for the second decoction with a duration of 0.5 h. The decoction of *P. rhizobium* praeparatum was prepared with the same method. Then the decoction of *A. kusnezoffii* reichb and *P. rhizobium* praeparatum was mixed. The filtered and mixed decoction was adjusted to 150 mL.

Mixed decoction of alcohol (MDA): The process was same as mixed decoction of water, but the reagent was substituted by alcohol rather than water.

Co-decoction decoctions of alcohol (CDA): The extract reagent was alcohol and the prepared process was same as co-decoction of water.

Each decoction was prepared with the same method for four times. Sixteen decoctions were centrifuged at 4000 rpm for 15 min and the supernatant was filtered through 0.22 μm membrane for UHPLC/Q-TOF-MS analysis.

UHPLC/Q-TOFMS conditions: The UHPLC analysis was carried out by Agilent 1200 Infinity (Agilent Corporation, Santa Clara, CA, USA) equipped with a quaternary pump, an online vacuum degas, an autosampler and an automatic thermostatic column oven. The separation was carried out on Poroshell ZORBAX Extend-C_{18} column (2.1 mm × 100 mm, 3.5 μm Agilent) at 30 ºC with a flow rate of 0.3 mL min^{-1} and injection volume was 5 μL. Mobile phase was a mixture of 0.1 % formic acid-water (A) and acetonitrile (B). The gradient program of mobile phase was carried out as follows: 0-1.5 min, 1-3 % B; 1.5-25 min, 3-30 % B; 15-25 min, 30-45 % B; 25-30 min, 45-99 % B; 30-32 min, 99-1 % B.

MS detection was conducted on an Agilent 6538 quadrupole time-of-flight mass spectrometer equipped with an electrospray ionization source (ESI). Ionization was performed in the positive electrospray mode. On the basis of the best response for most of the compounds, the final parameters were as follows: fragment (120V), Cap (4000V), nebulizer (40 psi), drying gas (N_2, 10 L/min, 350 ºC). The TOF-MS was calibrated daily, according to the manufacturers recommendations. The testing mass range was set from m/z 100-1000 with a scanning rate of 2 s^{-1}. Reference masses at m/z 121.05873 (purine, 1.125 × 10^{-6} mol/L, Agilent Corp.) and m/z 922.09798 (hexakis-(1H,1H,3H-tetrafluoropropoxy)-phosphazene, 5 × 10^{-6} mol/L, Agilent Corp.) was continually introduced, along with the LC stream for accurate mass calibration. The collision energy for each compound varied according to this formula: [5 × (mass/100)] +5.

Data processing and analysis: Dataset generated from the UHPLC-TOF/MS system were analyzed with Simca-P^+12.0 software. Data were analyzed with Orthogonal signal correction-partial least square discriminate analysis (OSC-PLS-DA). The score plot that reflects straggling extent of intergroup. The loading plot that reflects contribution range of ions. The trend plots that reflects ion's difference of each group were produced to accomplish the statistical analysis of experimental data.

RESULTS AND DISCUSSION

In water decoction, it was found that ions 590.2959 (11.29 min), 604.3113 (12.12 min) and 574.3006 (12.71 min) were detected with higher intensity in CDW, but much lower in MDW. Ions 486.2706 (4.58 min), 500.2856 (5.37 min) and 470.2749 (6.09 min) were detected with higher intensity in MDW, but were almost undetectable in CDW (Fig. 1a).

In alcohol decoction, it was also found that ions 632.3074 (14.49 min), 646.3225 (15.61 min), 616.3121 (15.74 min), 630.3274 (16.85 min) were detected with higher intensity in CDA than that in MDA and ions 486.2706 (4.58 min), 604.3120 (12.13 min), 574.3006 (12.71 min), 618.3272 (15.19 min), 602.3324 (16.45 min) were detected with higher intensity in MDA. These ions could be used as potential markers to distinguish MDA and CDA (Fig. 1b).

PLS-DA was applied together with a multivariate preprocessing filter called orthogonal signal correction (OSC) for developing a diagnostic tool. The OSC filter removes the uncorrelated signals resulting in information about the within-class variation. On the score plot (Fig. 2a,b), the experimental samples clearly clustered into two groups, the mixed decoction group and the co-decoction group, which indicates that the combination produced changes in the composition and/or content of elements in the decoction. To find the possible chemical markers between the mixed decoction group and co-decoction group, statistical analysis was performed to generate a loading plot (Fig. 2c,d). In the loading plot, each point with its intensity and its corresponding t_R-*m/z* pair information

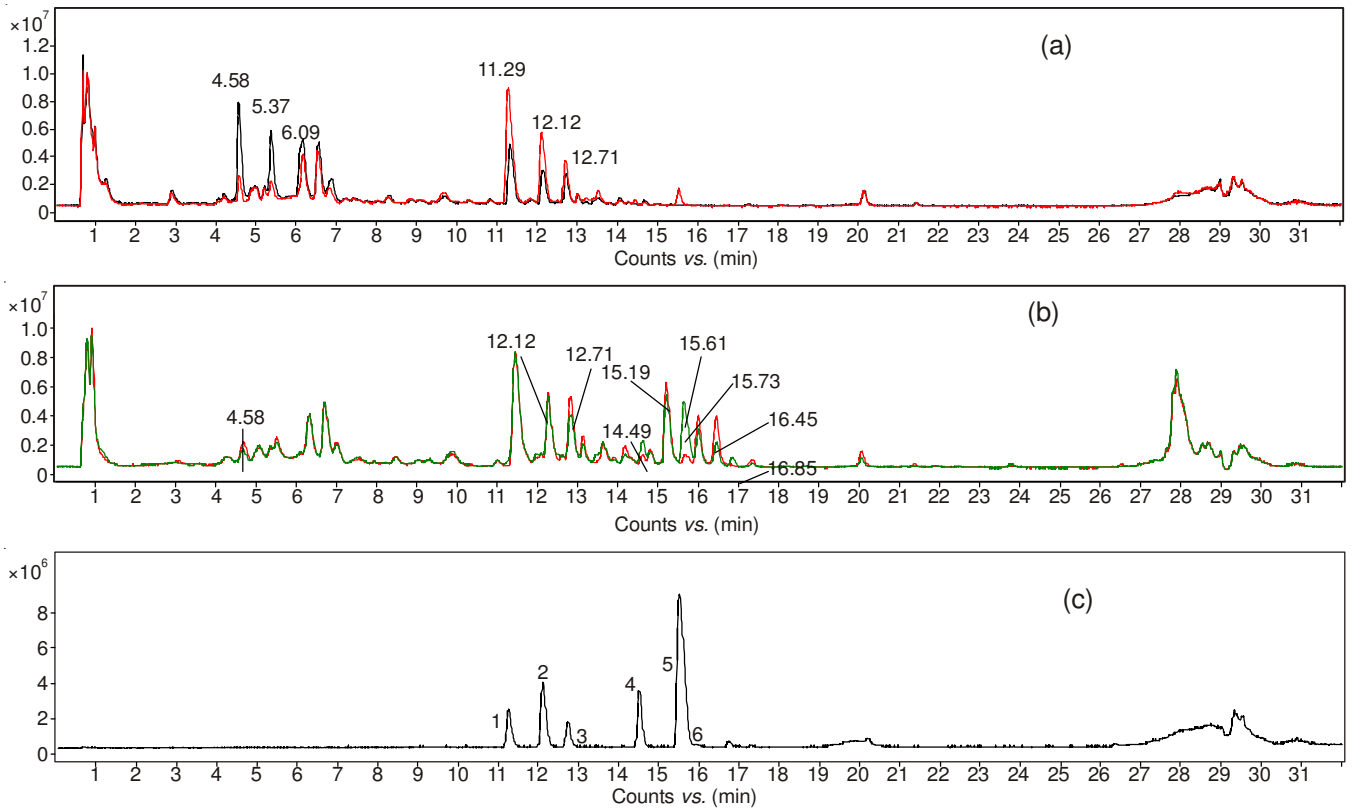
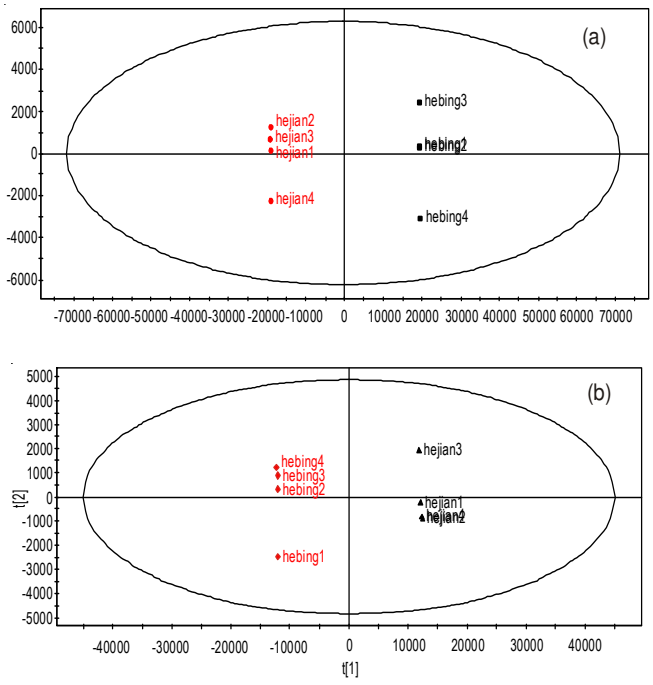


Fig. 1. Comparisons of LC-MS positive ions TIC graphs of CDW (Red) and MDW (black) (a), CDA (green) and MDA (red) of alcohol (b), the mixed standard solution(c) of benzoylmesaconine (1), benzoylaconine (2), benzoylhypaconine (3), mesaconitine (4), aconitine (5), hypaconitine (6)

represents a part. Chemical markers were selected according to the distance from the origin on the loading plot. The distances to the origin of a series of icons stand for the contribution of the variables in discrimination analysis model. The variables far away from the origin makes a greater contribution to the discrimination of the groups, so the components represented by these variables can be selected as potential chemical markers for the discrimination of the groups (Fig. 3).



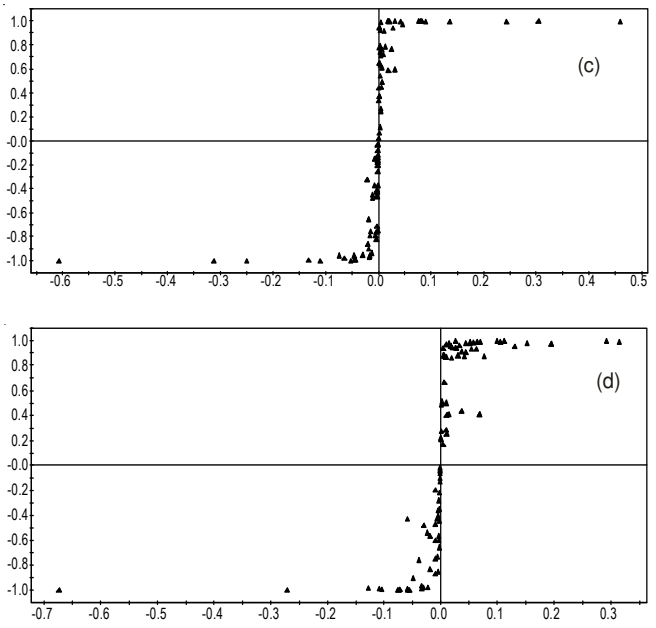


Fig. 2. Score scheme analysis on CDW and MDW of two drugs (a), CDA and MDA of two drugs (b) by OSC-PLS-DA. The loading diagram analysis on CDW and MDW of two drugs (c), CDA and MDA of two drugs (d) by OSC-PLS-DA

In the OSC-PLS-DA design (Fig. 3), the VIP value of the variable reflected the overall importance of the chemical components, which were closely related to their differentiation of the groups. In this study, the chemical components with VIP values greater than 1.0 were regarded as important markers.

TABLE-1
IDENTIFICATION IN POSITIVE IONS MODE ON MOLECULAR ION PEAK OF DIFFERENT COMPOUNDS BETWEEN CDW AND MDW OF TWO DRUGS

Peak No.	tR (min)	Assigned identity	m.f.	[M+H]+ (m/z)	Theoretical exact mass (Da)	Error of m/z (Da)	VIP
1	4.58	Mesaconine	$C_{24}H_{39}NO_9$	486.2706	486.26976	-1.7	4.5941
2	5.37	Aconine	$C_{25}H_{41}NO_9$	500.2856	500.28541	-0.4	3.0441
3	6.09	Hypaconine	$C_{24}H_{39}NO_8$	470.2749	470.27484	-0.1	2.4400
4	11.29	Benzoylmeaconine	$C_{31}H_{43}NO_{10}$	590.2959	590.29597	0.1	6.0614
5	12.12	Benzoylaconine	$C_{32}H_{45}NO_{10}$	604.3113	604.31162	0.5	3.1208
6	12.71	Benzoylhypaconine	$C_{31}H_{43}NO_9$	574.3006	574.30106	0.8	1.3206

TABLE-2
IDENTIFICATION IN POSITIVE IONS MODE ON MOLECULAR ION PEAK OF DIFFERENT COMPOUNDS BETWEEN CDA AND MDA OF TWO DRUGS

Peak No.	tR (min)	Assigned identity	m.f.	[M+H]+ (m/z)	Theoretical exact mass (Da)	Error of m/z (Da)	VIP
1	4.58	Mesaconine	$C_{24}H_{39}NO_9$	486.2706	486.26976	1.7	1.2043
2	12.12	Benzoylaconine	$C_{32}H_{45}NO_{10}$	604.3120	604.31162	0.6	1.3927
3	12.71	Benzoylhypaconine	$C_{31}H_{43}NO_9$	574.3006	574.30106	-0.8	3.3624
4	14.49	Mesaconitine	$C_{33}H_{45}NO_{11}$	632.3074	632.30654	1.4	1.0221
5	15.19	14-Benzoyl-8-O-methyl-aconine	$C_{32}H_{43}NO_{11}$	618.3272	618.3258	2.3	1.6227
6	15.61	Aconitine	$C_{34}H_{47}NO_{11}$	646.3225	646.32219	0.5	1.3594
7	15.73	Hypacontine	$C_{33}H_{45}NO_{10}$	616.3121	616.31162	0.8	6.2894
8	16.45	Neojiangyouaconitine	$C_{33}H_{47}NO_9$	602.3324	602.33236	0.1	3.1285
9	16.85	Deoxyaconitine	$C_{34}H_{47}NO_{10}$	630.3274	630.32727	0.2	1.0389

In water decoction, VIP value of BAC, BMA, BHA. aconine, mesaconine, hypaconine are larger than 1.0 (Table-1), so they are regarded as the important markers of water decoction. In alcohol decoction, AC, MA, HA, deoxyaconitine, 14-benzoyl-8-O-methyl-aconine, mesaconine, BMA, BHA, neojiangyouaconitine are considered to be the most important components (Table-2). Most of them are major components of *A. kusnezoffii* reichb.

AC, MA,HA, BAC, BMA, BHA were unambiguously identified by comparing their retention time with the mixed standard solution (Fig. 1c). Their accurate molecular mass data and fragmentation models can be identified by respective reference standards. The other compounds could only be tentatively assigned by comparing their differences in the fragmentation patterns and accurate mass data of their productions.

Discrimination of chemical constituents between co-decoctions and mixed decoctions may explore the mechanism of combination of *A. kusnezoffii* reichb and *P. rhizobium* praeparatum. The toxicity of *A. kusnezoffii* reichb mainly derives from DDAs that mainly includes AC, MA and HA. They can be hydrolyzed into less-toxic MDAs that including BAC, BMA, BHA, then MDAs can be hydrolyzed into few toxic amine alkaloids(AAs) that including aconine, mesaconine, hypaconine. The hydrolysis process of AC was showed in Fig. 4.

In water decoction, the content of DDAs of co-decoction and mixed decoction of water extract were low. The result was consistent with the other researchers' investigation. The content of MDAs including BAC, BMA, BHA in MDW was lower than that in CDW, but the content of AAs was higher in MDW, which means that the toxicity of mixed decoction dropped.

In alcohol decoction, the content of MDAs including AC, MA, HA, Deoxyaconitine in MDA was lower than that in CDA, but the content of 14-benzoyl-8-O-methyl-aconine,

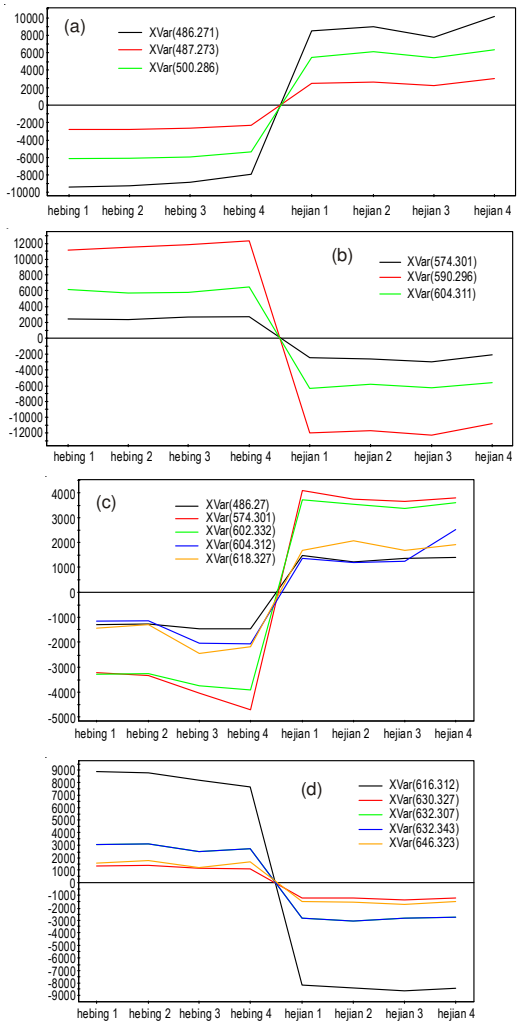


Fig. 3. Increased (a) and decreased (b) compounds analysis on CDW and MDW of two drugs by OSC-OPLS-DA.The increased (c) and decreased (d) compounds analysis on CDA and MDA of two drugs by OSC-OPLS-DA

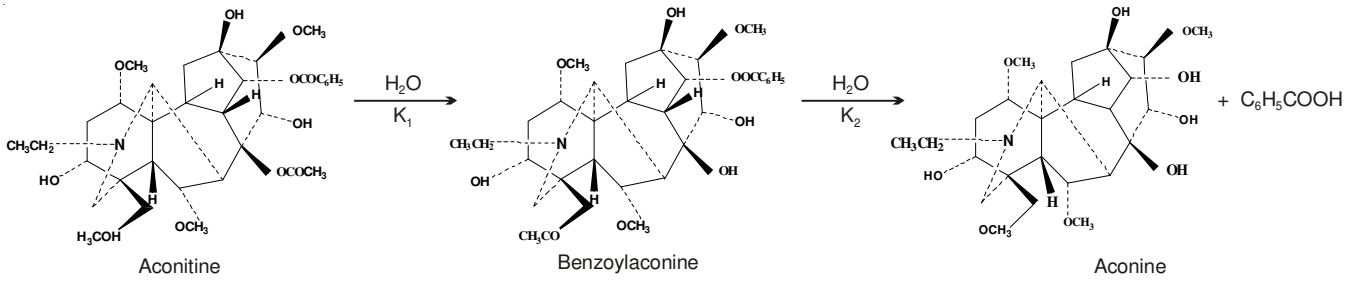


Fig. 4. Procedure of Aconitine's hydrolyzation

Mesaconine, BMA, BHA, Neojiangyouaconitine was lower in MDA, which means that the toxicity of mixed decoction dropped.

Under the direction of the theory of traditional Chinese medicine (TCM), the eighteen incompatible medications (Shi Ba Fan) have been proved to be important in enhancing the toxicity. However, in this paper, the content of BAC, BMA, BHA increased. The elevation of these constituents may promote the potency of the combination of *A. kusnezoffii* reichb and *P. rhizobium* praeparatum. On the other hand, AC, MA, HA, the representative toxic part of the herb, lowered significantly. To sum up, through a combination of *A. kusnezoffii* reichb and *P. rhizobium* praeparatum., the pharmacological activities could be enhanced while the side effects alleviated.

Based on these findings, the fact that it is the combination that caused changes of toxicity of chemical composition is confirmed. The exact mechanism of the combination is remains unclear and inconclusive. Therefore, more research is essential in the future.It is critical to study the differences between co-decoctions and mixed decoctions of *A. kusnezoffii* reichb and *P. rhizobium* praeparatum using metabolomics as a tool to investigate their metabolic profiles and to relate them to their different toxic effects. It is beneficial to guidance for clinical medication.

Conclusion

A strategy to screen out the possible chemical markers to discriminate combination by UHPLC/Q-TOF-MS coupled with multivariate statistical analysis was developed. *A. kusnezoffii* reichb and *P. rhizobium* praeparatum selected as a model herb to study the changes of metabolic profiling and detoxification of the combination. Moreover, explained mechanisms of the combination from the chemical composition, which may provide a scientific evidence to the combination of *A. kusnezoffii* reichb and *P. rhizobium* praeparatum in clinical treatment.

ACKNOWLEDGEMENTS

This work was financially supported by the National Basic Research Program of China (973 Program) (2011CB505302) The authors thank Dr. Qiyun Zhang for the valuable suggestions and support during the experiment.

REFERENCES

1. ChPC, Pharmacopoeia of People,s Republic of China, Beijing (2010).
2. D.M. Yu, Ph.D. Dissertation, Study on Compounding Rules of the Prescriptions with Aconite in Combination with Pinellia, Snakegourd Fruit, Fritillary, *Ampelopsis Japonica makino* or *Rhizoma bletillae*, Nanjing University of Chinese Medicine (2012).
3. G. Liu, *Chinese J. Ethnomed. Ethnopharm.*, **20**, 42 (2010).
4. K. Huang, W.H. Wang, B.C. Li, Q. Wang, J. Yang, W.Y. Li, *Res. Spectrosc. Lab.*, **26**, 922 (2009).
5. H. Yue, Z. Pi, H. Li, F. Song, Z. Liu and S. Liu, *Phytochem. Anal.*, **19**, 141 (2008).
6. D. Csupor, P. Forgo, E.M. Wenzig, R. Bauer and J. Hohmann, *J. Nat. Prod.*, **71**, 1779 (2008).
7. M. Liu, H. Zhang, L. Zhao, B. Zhao, L. Dong, Z. Zhu and Y. Chai, *J. Chromatogr. A*, **67**, 1003 (2008).
8. G. Liu, *China Foreign Medical*, **2**, 25 (2011).
9. S.L. Li, J.Z. Song, C.F. Qiao, Y. Zhou, K. Qian, K.H. Lee and H.X. Xu, *J. Pharm. Biomed. Anal.*, **51**, 812 (2010).